

ANNEEX

PATENT CLAIMS

1. A method for identification of biologically active nucleic acids or peptides or their cellular ligands, which comprises the steps of (a) production of a pool of appropriate vectors each containing a DNA sequence to be examined, (b) efficient transduction of said vectors into a number of identical eukaryotic cells in such a way that a single ribonucleic acid and possibly peptide is expressed or a limited number of different ribonucleic acids and peptides are expressed by each cell, (c) screening of said transduced cells to see whether some of them have changed a certain phenotypic trait, and (d) selection and cloning of said changed cells, characterized in that the pool of appropriate vectors in step (a) contain totally or partly random DNA sequences selected from the group consisting of:

i) synthetic totally random DNA sequences;

ii) synthetic random DNA sequences, in which restrictions upon the randomness may be introduced for the purpose of limiting the number of available sequences and/or for the introduction of post-translational modifications of expressed peptides;

iii) synthetic random DNA sequences like (i) or (ii) coupled to coding sequences of purification tags in order to facilitate the purification and identification of expressed peptides; and

iv) synthetic random DNA sequences like (ii), (iii) or (iii) coupled to the coding sequence of a protein;

and that either

5 (e) the vector DNA in the phenotypically changed cells is isolated and sequenced, and the sequences of the biologically active ribonucleic acids or peptides are deduced from the sequenced vector DNA;

or

10 10 (f) the biologically active ribonucleic acids or peptides expressed in the phenotypically changed cells are used directly for isolation of a ligand molecule to said ribonucleic acid or peptide.

15 15 2. A method according to claim 1, in which the peptide is a peptide sequence, introduced into or fused to a protein, preferably a F(ab) fragment or an antibody molecule.

20 20 3. A method according to claim 1 or 2; in which the amino acid sequences of the random peptide library are encoded by synthetic DNA sequences/oligonucleotides produced by codon split synthesis, where defined DNA codons are synthesized in a random order.

25 4. A method according to claim 1 or 2, in which the amino acid sequences of the random peptide library are encoded by synthetic DNA sequences/oligonucleotides produced by conventional random oligonucleotide synthesis.

30 30 5. A method according to any one of claims 1-4, in which the random DNA sequences are introduced into the expression vector by the principle of site directed PCR-mediated mutagenesis hereby ensuring the complexity of the library.

6. A method according to claim 5 in which 3'-5' exonuclease trimming of PCR product 3' ends is used for optimal combining efficiencies of two such PCR products.

5 7. A method according to any one of claims 1-6, in which temperature-cycling ligation is used for optimal ligation of a DNA fragment into a vector, maintaining a high diversity of the library for transfection into packaging cells.

10 8. A method according to any one of claims 1-7, in which the random DNA sequences are introduced into the number of eukaryotic cells in such a way that only one DNA sequence is introduced in each cell, one cell expressing 15 one ribonucleic acid and possibly one peptide, thus enabling a particular sequence to be isolated and analyzed.

15 9. A method according to any one of claims 1-8, in which the random DNA sequences are introduced into the eukaryotic cells by the use of appropriate viral vectors selected from e.g. retrovirus or vaccinia virus.

20 10. A method according to claim 9, in which the vector used is a retroviral vector.

25 11. A method according to claim 10, in which the retroviral vector has heterologous ends to facilitate PCR-based generation of the random DNA sequences.

30 12. A method according to claim 11, in which the heterologous ends contain two different promoters.

35 13. A method according to any one of claims 10-12, in which the retroviral vector contains a CMV promoter replacing the viral promoter in the 5'-LTR.

14. A method according to any one of claims 9-13, in which the random DNA sequences are produced as linear PCR products which are directly introduced into the virus packaging cells by non-viral transfection methods.

15. A method according to any one of claims 9-14, in which the viral DNA introduced into the cells is amplified directly by PCR and used for retransfection of new target cells with the purpose of eliminating false positives and/or enabling the "one cell - one ribonucleic acid or peptide" concept.

16. A method according to any one of claims 9-15, in which the viral titer of retroviral packaging cell lines is increased by transient transfection with a functional tRNA gene corresponding to the PBS in the vector.

17. A method according to any one of claims 9-16, in which a packaging cell line constructed from a vector expressing a single transcript translating the three polyproteins/proteins, gag-pol, a drug resistance gene, and the env gene is used.

18. A method according to any one of claims 9-17, in which a semi-packaging cell line with a corresponding minivirus/vector enabling vector expression after transduction rather than transfection of cells is used.

19. A method according to any one of claims 1-18, in which appropriate restrictions upon the random nature of the expressed peptides are introduced such as e.g. glycosylation sites and anchor residues.

20. A method according to any one of claims 1-19, in which the biologically active peptide or protein also

contains a purification tag enabling the direct isolation of the biologically active protein as well as the target protein causing the biological activity.

5 21. A method according to any one of claims 1-20, in which appropriate signal peptides, other leader molecules or recognition sequences also are encoded by the introduced DNA in such a way that they are fused to the expressed random peptides, or the expressed proteins containing the random peptide sequences, enabling these to be directed towards defined cellular compartments.

10 22. A method according to any one of claims 1-21, in which the random DNA sequences are introduced into, or fused to a DNA sequence encoding a protein expressed simultaneously from the library vectors.

15 23. A method according to claim 22, in which the protein is selected from the group consisting of secreted proteins, intracellular proteins, and membrane proteins e.g. signal transducing molecules.

20 24. A method according to claim 22 or 23, in which the protein is derived wholly or partly from the heavy and/or light chain of an antibody molecule.

25 25. A method according to any one of claims 1-24, which is used for identification of T cell epitopes.

30 26. A method according to any one of claims 1-24, which is used for identifying biologically active peptides which regulate cell surface expression of proteins.

27. Use of a ribonucleic acid or peptide identified by the method according to any one of claims 1-24 as a lead compound for drug development.

5 28. Use of a ribonucleic acid or peptide identified by the method according to any one of claims 1-24 for isolation of a cellular ligand interacting with said ribonucleic acid or peptide.

10 29. Use of a protein containing a particular amino acid sequence identified by the method according to any one of claims 1-24 for isolation of a cellular ligand interacting with said particular amino acid sequence contained in said protein.

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